AD NUMBER

AD819932

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AD

TECHNICAL MANUSCRIPT 390

MICRODIFFUSION IODINATION OF PURIFIED MICROBIAL ANTIGENS FOR COPRECIPITATION SEROLOGY

Jack Gruber
George G. Wright

JULY 1967

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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Jack Gruber

George G. Wright

Medical Investigation Division
MEDICAL SCIENCES LABORATORY

Project 1C014501B71A

July 1967

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

Conventional methods for introduction of I^{131} may modify or destroy the serological reactivity of labile microbial antigens. A modified microdiffusion iodination method that eliminates the exposure of antigens to excess oxidizing agent produced satisfactory trace labeling of purified protective antigen of Bacillus anthracis and enterotoxin B of Staphylococcus aureus without detectable change in precipitating activity. The iodinated antigens proved suitable for use in the ammonium sulfate coprecipitation technique of Farr.

MICRODIFFUSION IODINATION OF PURIFIED MICROBIAL ANTIGENS FOR COPRECIPITATION SEROLOGY

Although combining power for antigen is the fundamental expression of antibody activity, most quantitative serological methods measure only secondary effects of such combination. Accordingly, these methods may provide inaccurate estimates of antigen combining power and inadequate tests of the significance of specific antibodies in acquired resistance to infection. The ammonium sulfate coprecipitation technique of Farr,[1] using antigen labeled with iodine^{131}, provides a quantitative and sensitive measure of the capacity of antisera to bind protein antigens. Despite the sensitivity and precision of the method,[2] it has been virtually neglected by those studying resistance to infection, probably in part because of difficulty in iodinating relatively labile microbial antigens. During a study of the application of coprecipitation serology to certain purified microbial antigens,[3] a microdiffusion procedure was employed for trace iodination. This method provided labeled antigens suitable for serological use and should facilitate the application of Farr's method to other antigen-antibody systems.

Protective antigen of Bacillus anthracis, prepared in our laboratory,[4] and enterotoxin B of Staphylococcus aureus, kindly supplied by Dr. E.J. Schantz,[5] were investigated. Attempts to iodinate the purified anthrax protective antigen by the direct nitrous acid procedure[6] did not yield suitably labeled material that retained serological activity. Use of chloramine-T[7] and other procedures also failed to yield suitable material. Because of this difficulty a modification of the microdiffusion procedure of Banerjee and Ekins[8] was investigated. This technique involves transfer of iodine to the protein solution by gaseous diffusion and eliminates addition of excess oxidizing agents to the protein.

The modification employed was similar to that described by Seth for iodination of serum globulin.[9] In place of a Conway microdiffusion cell, a modified 50-ml Erlenmeyer flask was used. A section of glass tubing 2.5 cm high and 1 cm in diameter was sealed to the bottom of the flask, thus forming a separate inner compartment. The protein solution to be iodinated, usually 1 ml, was pipetted into the outer chamber of the vessel. Two-tenths milliliter of 0.002 M KI was pipetted into the central compartment, 50 to 100 μc of I^{131} (as NaI^{131}) were added, and the flask was sealed with a skirt-type vaccine stopper. Stock acid dichromate solution (0.27 M $Na_2Cr_2O_7$ in 36 N H_2SO_4) was diluted 1:20, and 0.2 ml was added to the central compartment by a syringe with attached 3- or 4-inch needle. A large excess of oxidizing agent must be avoided; otherwise the iodine will be further oxidized to iodate. The flask was held at room temperature (23 to 25 C) and gently rotated occasionally. After 1 hour the labeled protein solution was withdrawn with another syringe and needle. The free iodide was removed by passage through a Sephadex G-25 column or by dialysis.

In studies with the purified anthrax protective antigen (1 mg per ml), 80% of the radioiodide was converted to gaseous iodine and approximately 20% of the total iodine was transferred to the protein solution. The transferred iodine bound to antigen ranged from 10 to 35%. Usually, about 1 atom of iodine was bound per molecule of antigen. Iodination resulting in labeling below the level of 4 atoms of iodine per molecule of antigen did not affect the precipitating activity of the antigen; labeling above the level of 7 atoms per molecule caused appreciable destruction.

In studies with the purified enterotoxin B (2 mg per ml), 70 to 90% of the initial radioiodide was converted to gaseous iodine and approximately 25% was transferred to the toxin. Efficiency of labeling averaged approximately 15% of the transferred iodine. Usually 0.5 to 1.5 atoms of iodine were bound per molecule of toxin. Both an Ouchterlony plate titration and a modified Oudin procedure[10] indicated that essentially all of the precipitating activity was retained after iodination and dialysis.

With both antigens, iodination by microdiffusion resulted in labeled material suitable for use in the ammonium sulfate coprecipitation technique. Tests with the labeled anthrax protective antigen revealed marked differences in the combining capacities of various equine sera (Fig. 1). Hyperimmune pony sera at 1:10 dilution precipitated 86 to 98% of the labeled antigen preparation, although only 7% was precipitated by a 1:10 dilution of normal horse serum. Differences in immune sera were evident and were characterized by determining the dilution of serum required to precipitate 50% of the antigen. The reciprocal of that serum dilution represented the titer of the serum. Labeled enterotoxin B also proved suitable for use in coprecipitation serology. Using 1.6 M ammonium sulfate for coprecipitation, differences were found in the combining capacities of normal and immune rabbit sera (Fig. 2).

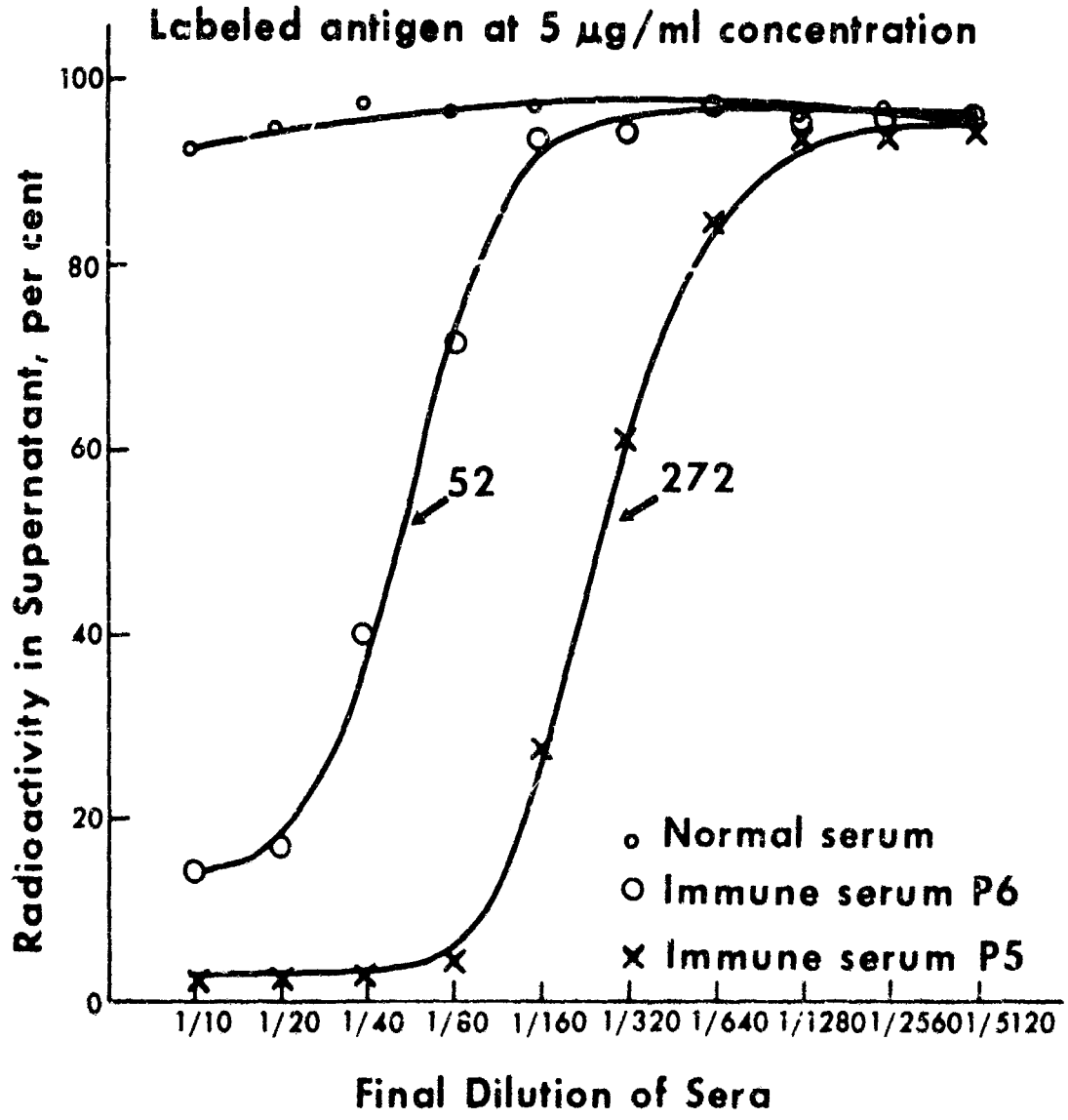

Figure 1. Coprecipitation of Labeled Anthrax Protective Antigen 4048 and Equine Sera by 1.4 M Ammonium Sulfate.

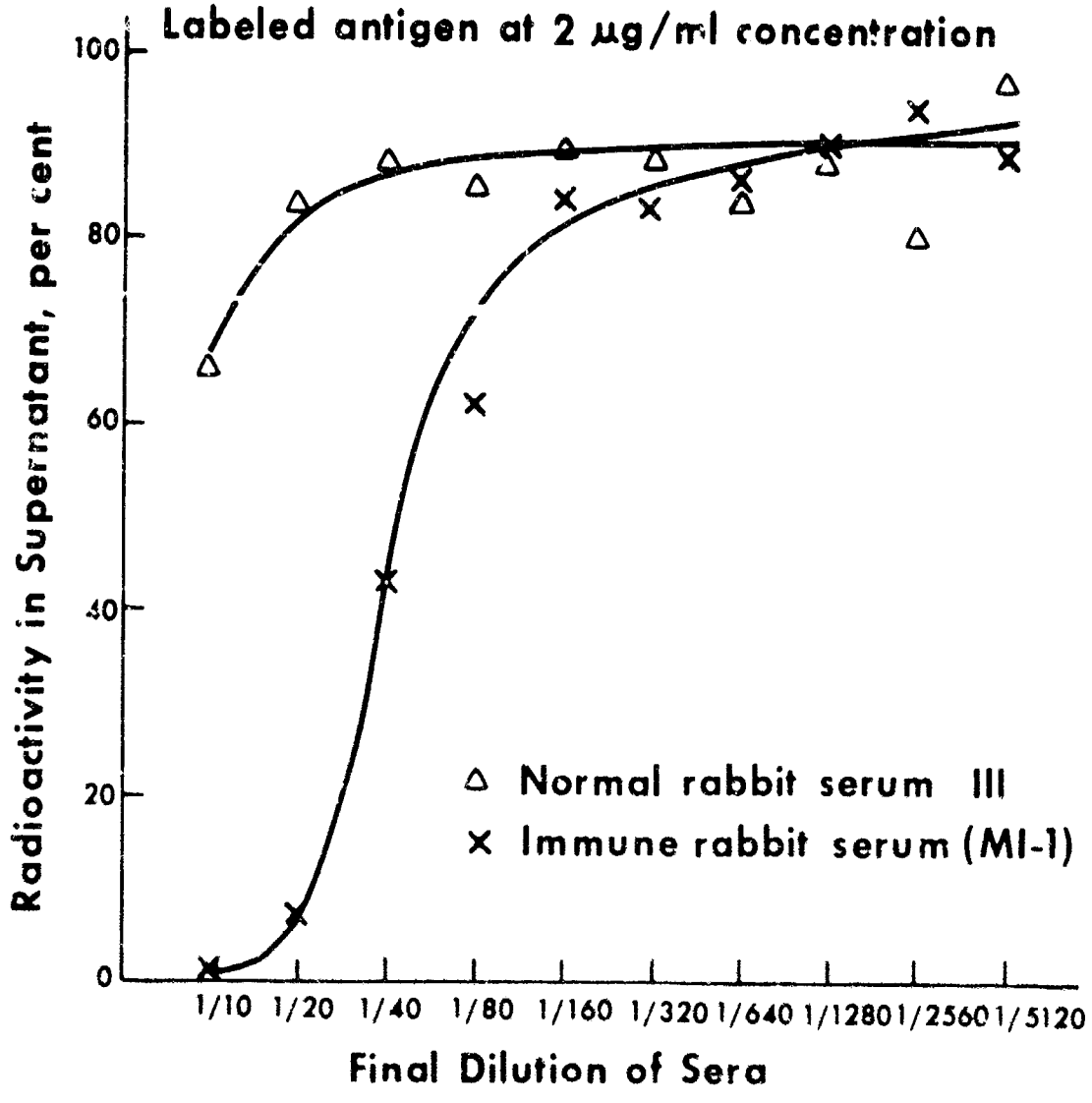

Figure 2. Coprecipitation of Labeled Enterotoxin B and Rabbit Sera Using 1.6 M Ammonium Sulfate.

SUMMARY

Conventional methods for introduction of I^{131} may modify or destroy the serological reactivity of labile microbial antigens. A modified microdiffusion iodination method that eliminates the exposure of antigens to excess oxidizing agent produced satisfactory trace labeling of purified protective antigen of Bacillus anthracis and enterotoxin B of Staphylococcus aureus without detectable change in precipitating activity. The iodinated antigens proved suitable for use in the ammonium sulfate coprecipitation technique of Farr.

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Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*

Department of the Army
Fort Detrick, Frederick, Maryland, 21701

2a. REPORT SECURITY CLASSIFICATION

Unclassified

2b. GROUP

3. REPORT TITLE

MICRODIFFUSION IODINATION OF PURIFIED MICROBIAL ANTIGENS FOR COPRECIPITATION SEROLOGY

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5. AUTHOR(S) *(First name, middle initial, last name)*

Gruber, Jack (NMI)
Wright, George G.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
July 1967	10	10

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1C014501B71A	Technical Manuscript 390
c.	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Department of the Army Fort Detrick, Frederick, Maryland, 21701

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14. Key Words

*Antigens
*Microdiffusion
Bacillus anthracis
Staphylococcus aureus
Radioactive
Iodine-131

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